Human Codon-Optimized HER2/neu Nucleotide Sequence

```
   1 ATGGAGCTGG CCGCCCTGTG CCGCTGGGGC CTGCTGCTGG CCCTGCTGCC
  51 CCCCGGCGCC GCCAGCACCC AGGTGTGCAC CGGCACCGAC ATGAAGCTGC
 101 GCCTGCCCGC CAGCCCCGAG ACCCACCTGG ACATGCTGCG CCACCTGTAC
 151 CAGGGCTGCC AGGTGGTGCA GGGCAACCTG GAGCTGACCT ACCTGCCCAC
 201 CAACGCCAGC CTGAGCTTCC TGCAGGACAT CCAGGAGGTG CAGGGCTACG
 251 TGCTGATCGC CCACAACCAG GTGCGCCAGG TGCCCCTGCA GCGCCTGCGC
 301 ATCGTGCGCG GCACCCAGCT GTTCGAGGAC AACTACGCCC TGGCCGTGCT
 351 GGACAACGGC GACCCCCTGA ACAACACCAC CCCCGTGACC GGCGCCAGCC
 401 CCGGCGGCCT GCGCGAGCTG CAGCTGCGCA GCCTGACCGA GATCCTGAAG
 451 GGCGGCGTGC TGATCCAGCG CAACCCCCAG CTGTGCTACC AGGACACCAT
 501 CCTGTGGAAG GACATCTTCC ACAAGAACAA CCAGCTGGCC CTGACCCTGA
 551 TCGACACCAA CCGCAGCCGC GCCTGCCACC CCTGCAGCCC CATGTGCAAG
 601 GGCAGCCGCT GCTGGGGCGA GAGCAGCGAG GACTGCCAGA GCCTGACCCG
 651 CACCGTGTGC GCCGGCGGCT GCGCCCGCTG CAAGGGCCCC CTGCCCACCG
 701 ACTGCTGCCA CGAGCAGTGC GCCGCCGGCT GCACCGGCCC CAAGCACAGC
 751 GACTGCCTGG CCTGCCTGCA CTTCAACCAC AGCGGCATCT GCGAGCTGCA
 801 CTGCCCCGCC CTGGTGACCT ACAACACCGA CACCTTCGAG AGCATGCCCA
 851 ACCCCGAGGG CCGCTACACC TTCGGCGCCA GCTGCGTGAC CGCCTGCCCC
 901 TACAACTACC TGAGCACCGA CGTGGGCAGC TGCACCCTGG TGTGCCCCCT
 951 GCACAACCAG GAGGTGACCG CCGAGGACGG CACCCAGCGC TGCGAGAAGT
1001 GCAGCAAGCC CTGCGCCCGC GTGTGCTACG GCCTGGGCAT GGAGCACCTG
1051 CGCGAGGTGC GCGCCGTGAC CAGCGCCAAC ATCCAGGAGT TCGCCGGCTG
1101 CAAGAAGATC TTCGGCAGCC TGGCCTTCCT GCCCGAGAGC TTCGACGGCG
1151 ACCCCGCCAG CAACACCGCC CCCCTGCAGC CCGAGCAGCT GCAGGTGTTC
1201 GAGACCCTGG AGGAGATCAC CGGCTACCTG TACATCAGCG CCTGGCCCGA
1251 CAGCCTGCCC GACCTGAGCG TGTTCCAGAA CCTGCAGGTG ATCCGCGGCC
1301 GCATCCTGCA CAACGGCGCC TACAGCCTGA CCCTGCAGGG CCTGGGCATC
1351 AGCTGGCTGG GCCTGCGCAG CCTGCGCGAG CTGGGCAGCG GCCTGGCCCT
1401 GATCCACCAC AACACCCACC TGTGCTTCGT GCACACCGTG CCCTGGGACC
1451 AGCTGTTCCG CAACCCCCAC CAGGCCCTGC TGCACACCGC CAACCGCCCC
1501 GAGGACGAGT GCGTGGGCGA GGGCCTGGCC TGCCACCAGC TGTGCGCCCG
1551 CGGCCACTGC TGGGGCCCCG GCCCCACCCA GTGCGTGAAC TGCAGCCAGT
1601 TCCTGCGCGG CCAGGAGTGC GTGGAGGAGT GCCGCGTGCT GCAGGGCCTG
1651 CCCCGCGAGT ACGTGAACGC CCGCCACTGC CTGCCCTGCC ACCCCGAGTG
1701 CCAGCCCCAG AACGGCAGCG TGACCTGCTT CGGCCCCGAG GCCGACCAGT
1751 GCGTGGCCTG CGCCCACTAC AAGGACCCCC CCTTCTGCGT GGCCCGCTGC
1801 CCCAGCGGCG TGAAGCCCGA CCTGAGCTAC ATGCCCATCT GGAAGTTCCC
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FIG. 1A

Human HER2/neu Protein Sequence

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   1 MELAALCRWG LLLALLPPGA ASTQVCTGTD MKLRLPASPE THLDMLRHLY QGCQVVQGNL
  61 ELTYLPTNAS LSFLQDIQEV QGYVLIAHNQ VRQVPLQRLR IVRGTQLFED NYALAVLDNG
 121 DPLNNTTPVT GASPGGLREL QLRSLTEILK GGVLIQRNPQ LCYQDTILWK DIFHKNNQLA
 181 LTLIDTNRSR ACHPCSPMCK GSRCWGESSE DCQSLTRTVC AGGCARCKGP LPTDCCHEQC
 241 AAGCTGPKHS DCLACLHFNH SGICELHCPA LVTYNTDTFE SMPNPEGRYT FGASCVTACP
 301 YNYLSTDVGS CTLVCPLHNQ EVTAEDGTQR CEKCSKPCAR VCYGLGMEHL REVRAVTSAN
 361 IQEFAGCKKI FGSLAFLPES FDGDPASNTA PLQPEQLQVF ETLEEITGYL YISAWPDSLP
 421 DLSVFQNLQV IRGRILHNGA YSLTLQGLGI SWLGLRSLRE LGSGLALIHH NTHLCFVHTV
 481 PWDQLFRNPH QALLHTANRP EDECVGEGLA CHQLCARGHC WGPGPTQCVN CSQFLRGQEC
 541 VEECRVLQGL PREYVNARHC LPCHPECQPQ NGSVTCFGPE ADQCVACAHY KDPPFCVARC
 601 PSGVKPDLSY MPIWKFPDEE GACQPCPINC THSCVDLDDK GCPAEQRASP LTSIISAVVG
 661 ILLVVVLGVV FGILIKRRQQ KIRKYTMRRL LQETELVEPL TPSGAMPNQA QMRILKETEL
 721 RKVKVLGSGA FGTVYKGIWI PDGENVKIPV AIAVLRENTS PKANKEILDE AYVMAGVGSP
 781 YVSRLLGICL TSTVQLVTQL MPYGCLLDHV RENRGRLGSQ DLLNWCMQIA KGMSYLEDVR
 841 LVHRDLAARN VLVKSPNHVK ITDFGLARLL DIDETEYHAD GGKVPIKWMA LESILRRRFT
 901 HQSDVWSYGV TVWELMTFGA KPYDGIPARE IPDLLEKGER LPQPPICTID VYMIMVKCWM
 961 IDSECRPRFR ELVSEFSRMA RDPQRFVVIQ NEDLGPASPL DSTFYRSLLE DDDMGDLVDA
1021 EEYLVPQQGF FCPDPAPGAG.GMVHHRHRSS STRSGGGDLT LGLEPSEEEA PRSPLAPSEG
1081 AGSDVFDGDL-GMGAAKGLQS.LPTHDPSPLQ RYSEDPTVPL PSETDGYVAP LTCSPQPEYV
1141 NQPDVRPQPP SPREGPLPAA RPAGATLERP KTLSPGKNGV VKDVFAFGGA VENPEYLTPQ
1201 GGAAPQPHPP PAFSPAFDNL YYWDQDPPER GAPPSTFKGT PTAENPEYLG LDVPV*
```

(SEQ ID NO:2)

FIG.1B

IMMUNODOMINANT T-CELL EPITOPES IN HUMAN HER2/neu PROTEIN

		IFN-γ ELIspot		IFN-γ INTRACELLULAR STAINING BALB/c		NeuT	
		BALB/c	NeuT	$CD4^+$	$CD8^+$	$CD4^+$	$CD8^+$
hNeu-1 TO hNeu 30 (aa 1-131)	POOL A	1,127					
hNeu-11 TO hNeu-15 (aa 41-71)	SUBPOOL A_{III}	1,291					
hNeu-16 TO hNeu-20 (aa 61-91)	SUBPOOL A_{IV}	1,057					
	hNeu15	1,095	1,289	0.27	46.35	0.25	47.78
	hNeu16	1,075		0.24	42.43		
	hNeu15.1	518	674	0.15	25.67	0.15	25.70
	hNeu15.2	143	265	0.17	1.82	0.05	3.36
	hNeu15.3	1,258	1,488	0.22	48.78	0.39	47.43
Q G N L E L T Y L P T N A S L S F L Q							
(SEC ID NO:5)							
hNeu-31 TO hNeu-60 (aa 121-251)	POOL B	65	n.t.			n.t.	n.t.
hNeu-41 TO hNeu-45 (aa 161-191)	SUBPOOL B_{III}	81	n.t.			n.t.	n.t.
	hNeu41	32	30	0.35	0.23	0.36	0.24
	hNeu42	42	35	0.47	0.23	0.34	0.23
161 L C Y Q D T I L W K D I F H K N N Q L							
(SEC ID NO:6)							
hNeu-301 TO hNeu-311 (aa 1201-1255)	POOL K	150	n.t.			n.t	n.t
hNeu-301 TO hNeu-305 (aa 1201-1231)	SUBPOOL K_I	165	n.t.			n.t.	n.t.
	hNeu301	128	114	0.24	3.04	0.17	5.71
1202 G G A A P Q P H P P P A F S P							
(SEC ID NO:7)							
	DMSO			0.10	0.12	0.18	0.17
	SEB			1.04	2.07	1.11	1.56

FIG.2

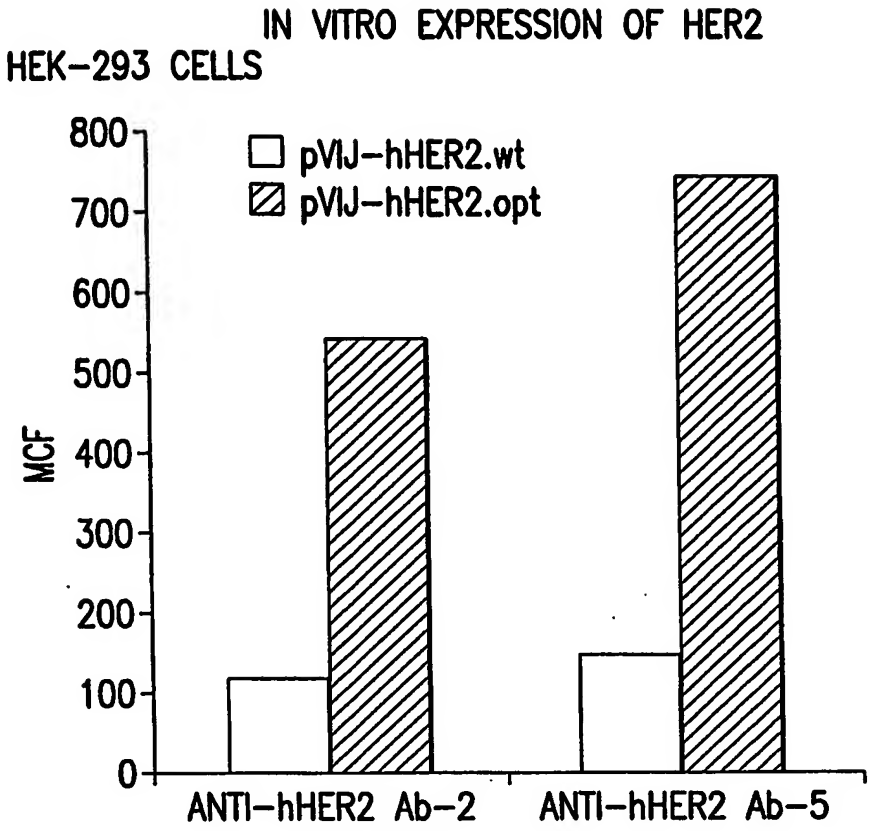
IN VITRO EXPRESSION OF HER2
HEK-293 CELLS
pVIJ-hHER2.wt
pVIJ-hHER2.opt
MCF
800
700
600
500
400
300
200
100
0
ANTI-hHER2 Ab-2
ANTI-hHER2 Ab-5

FIG.3A

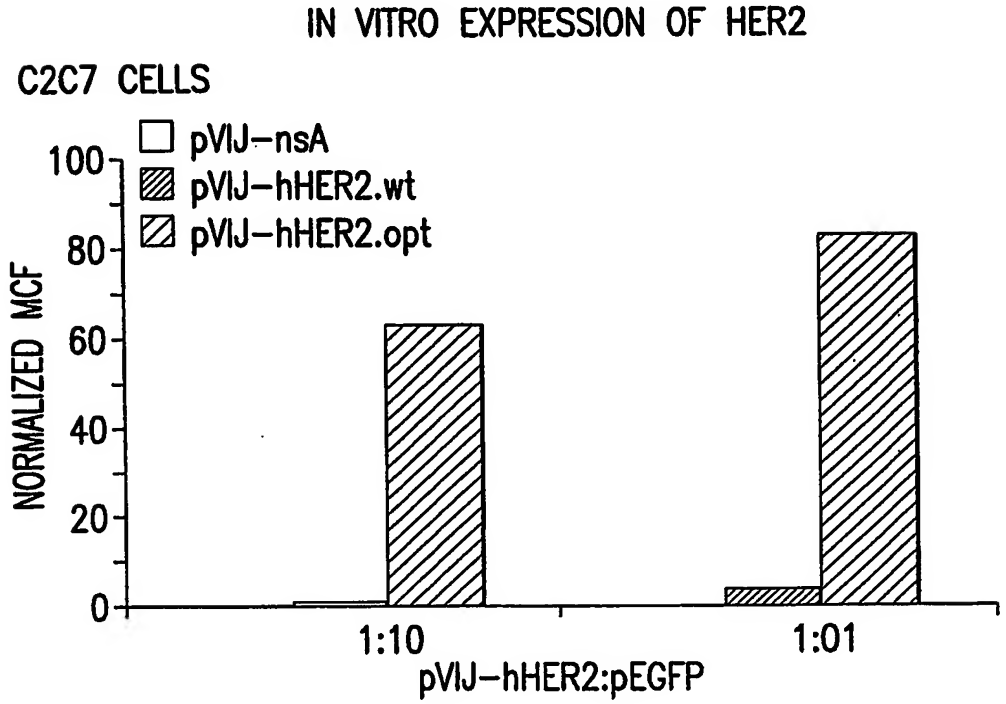
IN VITRO EXPRESSION OF HER2
C2C7 CELLS
pVIJ-nsA
pVIJ-hHER2.wt
pVIJ-hHER2.opt
NORMALIZED MCF
100
80
60
40
20
0
1:10
1:01
pVIJ-hHER2:pEGFP

FIG.3B

IMMUNE RESPONSE TO HUMAN HER2

MOUSE IFNγELISPOT		PEPTIDE	
	MOUSE#	hNeu15.3 (CD8+)	hNeu42 (CD4+)
pV1J_hHER2.wt	14, 17	15	2
pV1J_hHER2.opt	1, 8	286	48
pV1J_hHER2wt	3, 7	28	16
pV1J_hHER2.opt	2, 5	250	60

FIG.4A

IMMUNE RESPONSE TO HUMAN HER2

ISOTYPING ANTI-humHER2 Ab

	IgG1	IgG2a
pV1J_hHER2.wt	<100	<100
pV1J_hHER2.opt	45,940	77,648

FIG.4B

IMMUNIZATION OF MICE WITH pV1J-HER2 AND Ad5-hHER2

	Ad5-hHER2		pV1J-hHER2 w/ES	
	BALB/c	NeuT	BALB/c	NeuT
hNeu15.3	1,258	1,488	41	56
hNeu41	32	30	1	2
hNeu301	128	114	37	30

FIG.5

Human Codon-Optimized HER2ECDTM Nucleotide Sequence

ATGGAGCTGG CCGCCCTGTG CCGCTGGGGC CTGCTGCTGG CCCTGCTGCC CCCCGGCGCC
GCCAGCACCC AGGTGTGCAC CGGCACCGAC ATGAAGCTGC GCCTGCCCGC CAGCCCCGAG
ACCCACCTGG ACATGCTGCG CCACCTGTAC CAGGGCTGCC AGGTGGTGCA GGGCAACCTG
GAGCTGACCT ACCTGCCCAC CAACGCCAGC CTGAGCTTCC TGCAGGACAT CCAGGAGGTG
CAGGGCTACG TGCTGATCGC CCACAACCAG GTGCGCCAGG TGCCCCTGCA GCGCCTGCGC
ATCGTGCGCG GCACCCAGCT GTTCGAGGAC AACTACGCCC TGGCCGTGCT GGACAACGGC
GACCCCCTGA ACAACACCAC CCCCGTGACC GGCGCCAGCC CCGGCGGCCT GCGCGAGCTG
CAGCTGCGCA GCCTGACCGA GATCCTGAAG GGCGGCGTGC TGATCCAGCG CAACCCCCAG
CTGTGCTACC AGGACACCAT CCTGTGGAAG GACATCTTCC ACAAGAACAA CCAGCTGGCC
CTGACCCTGA TCGACACCAA CCGCAGCCGC GCCTGCCACC CCTGCAGCCC CATGTGCAAG
GGCAGCCGCT GCTGGGGCGA GAGCAGCGAG GACTGCCAGA GCCTGACCCG CACCGTGTGC
GCCGGCGGCT GCGCCCGCTG CAAGGGCCCC CTGCCCACCG ACTGCTGCCA CGAGCAGTGC
GCCGCCGGCT GCACCGGCCC CAAGCACAGC GACTGCCTGG CCTGCCTGCA CTTCAACCAC
AGCGGCATCT GCGAGCTGCA CTGCCCCGCC CTGGTGACCT ACAACACCGA CACCTTCGAG
AGCATGCCCA ACCCCGAGGG CCGCTACACC TTCGGCGCCA GCTGCGTGAC CGCCTGCCCC
TACAACTACC TGAGCACCGA CGTGGGCAGC TGCACCCTGG TGTGCCCCCT GCACAACCAG
GAGGTGACCG CCGAGGACGG CACCCAGCGC TGCGAGAAGT GCAGCAAGCC CTGCGCCCGC
GTGTGCTACG GCCTGGGCAT GGAGCACCTG CGCGAGGTGC GCGCCGTGAC CAGCGCCAAC
ATCCAGGAGT TCGCCGGCTG CAAGAAGATC TTCGGCAGCC TGGCCTTCCT GCCCGAGAGC
TTCGACGGCG ACCCCGCCAG CAACACCGCC CCCCTGCAGC CCGAGCAGCT GCAGGTGTTC
GAGACCCTGG AGGAGATCAC CGGCTACCTG TACATCAGCG CCTGGCCCGA CAGCCTGCCC
GACCTGAGCG TGTTCCAGAA CCTGCAGGTG ATCCGCGGCC GCATCCTGCA CAACGGCGCC
TACAGCCTGA CCCTGCAGGG CCTGGGCATC AGCTGGCTGG GCCTGCGCAG CCTGCGCGAG
CTGGGCAGCG GCCTGGCCCT GATCCACCAC AACACCCACC TGTGCTTCGT GCACACCGTG
CCCTGGGACC AGCTGTTCCG CAACCCCCAC CAGGCCCTGC TGCACACCGC CAACCGCCCC
GAGGACGAGT GCGTGGGCGA GGGCCTGGCC TGCCACCAGC TGTGCGCCCG CGGCCACTGC
TGGGGCCCCG GCCCCACCCA GTGCGTGAAC TGCAGCCAGT TCCTGCGCGG CCAGGAGTGC
GTGGAGGAGT GCCGCGTGCT GCAGGGCCTG CCCCGCGAGT ACGTGAACGC CCGCCACTGC
CTGCCCTGCC ACCCCGAGTG CCAGCCCCAG AACGGCAGCG TGACCTGCTT CGGCCCCGAG
GCCGACCAGT GCGTGGCCTG CGCCCACTAC AAGGACCCCC CCTTCTGCGT GGCCCGCTGC
CCCAGCGGCG TGAAGCCCGA CCTGAGCTAC ATGCCCATCT GGAAGTTCCC CGACGAGGAG
GGCGCCTGCC AGCCCTGCCC CATCAACTGC ACCCACAGCT GCGTGGACCT GGACGACAAG
GGCTGCCCCG CCGAGCAGCG CGCCAGCCCC CTGACCAGCA TCATCAGCGC CGTGGTGGGC
ATCCTGCTGG TGGTGGTGCT GGGCGTGGTG TTCGGCATCC TGATCTGA (SEQ ID NO:9)

FIG.6A

Human HER2ECDTM wt Nucleotide Sequence

ATGGAGCTG GCGGCCTTG TGCCGCTGG GGGCTCCTC CTCGCCCTC TTGCCCCCC GGAGCCGCG
AGCACCCAA GTGTGCACC GGCACAGAC ATGAAGCTG CGGCTCCCT GCCAGTCCC GAGACCCAC
CTGGACATG CTCCGCCAC CTCTACCAG GGCTGCCAG GTGGTGCAG GGAAACCTG GAACTCACC
TACCTGCCC ACCAATGCC AGCCTGTCC TTCCTGCAG GATATCCAG GAGGTGCAG GGCTACGTG
CTCATCGCT CACAACCAA GTGAGGCAG GTCCCACTG CAGAGGCTG CGGATTGTG CGAGGCACC
CAGCTCTTT GAGGACAAC TATGCCCTG GCCGTGCTA GACAATGGA GACCCGCTG AACAATACC
ACCCCTGTC ACAGGGGCC TCCCCAGGA GGCCTGCGG GAGCTGCAG CTTCGAAGC CTCACAGAG
ATCTTGAAA GGAGGGGTC TTGATCCAG CGGAACCCC CAGCTCTGC TACCAGGAC ACGATTTTG
TGGAAGGAC ATCTTCCAC AAGAACAAC CAGCTGGCT CTCACACTG ATAGACACC AACCGCTCT
CGGGCCTGC CACCCCTGT TCTCCGATG TGTAAGGGC TCCCGCTGC TGGGGAGAG AGTTCTGAG
GATTGTCAG AGCCTGACG CGCACTGTC TGTGCCGGT GGCTGTGCC CGCTGCAAG GGGCCACTG
CCCACTGAC TGCTGCCAT GAGCAGTGT GCTGCCGGC TGCACGGGC CCCAAGCAC TCTGACTGC
CTGGCCTGC CTCCACTTC AACCACAGT GGCATCTGT GAGCTGCAC TGCCCAGCC CTGGTCACC
TACAACACA GACACGTTT GAGTCCATG CCCAATCCC GAGGCCCGG TATACATTC GGCGCCAGC
TGTGTGACT GCCTGTCCC TACAACTAC CTTTCTACG GACGTGGGA TCCTGCACC CTCGTCTGC
CCCCTGCAC AACCAAGAG GTGACAGCA GAGGATGGA ACACAGCGG TGTGAGAAG TGCAGCAAG
CCCTGTGCC CGAGTGTGC TATGGTCTG GGCATGGAG CACTTGCGA GAGGTGAGG GCAGTTACC
AGTGCCAAT ATCCAGGAG TTTGCTGGC TGCAAGAAG ATCTTTGGG AGCCTGGCA TTTCTGCCG
GAGAGCTTT GATGGGGAC CCAGCCTCC AACACTGCC CCGCTCCAG CCAGAGCAG CTCCAAGTG
TTTGAGACT CTGGAAGAG ATCACAGGT TACCTATAC ATCTCAGCA TGGCCGGAC AGCCTGCCT
GACCTCAGC GTCTTCCAG AACCTGCAA GTAATCCGG GGACGAATT CTGCACAAT GGCGCCTAC
TCGCTGACC CTGCAAGGG CTGGGCATC AGCTGGCTG GGGCTGCGC TCACTGAGG GAACTGGGC
AGTGGACTG GCCCTCATC CACCATAAC ACCCACCTC TGCTTCGTG CACACGGTG CCCTGGGAC
CAGCTCTTT CGGAACCCG CACCAAGCT CTGCTCCAC ACTGCCAAC CGGCCAGAG GACGAGTGT
GTGGGCGAG GGCCTGGCC TGCCACCAG CTGTGCGCC CGAGGGCAC TGCTGGGGT CCAGGGCCC
ACCCAGTGT GTCAACTGC AGCCAGTTC CTTCGGGGC CAGGAGTGC GTGGAGGAA TGCCGAGTA
CTGCAGGGG CTCCCCAGG GAGTATGTG AATGCCAGG CACTGTTTG CCGTGCCAC CCTGAGTGT
CAGCCCCAG AATGGCTCA GTGACCTGT TTTGGACCG GAGGCTGAC CAGTGTGTG GCCTGTGCC
CACTATAAG GACCCTCCC TTCTGCGTG GCCCGCTGC CCCAGCGGT GTGAAACCT GACCTCTCC
TACATGCCC ATCTGGAAG TTTCCAGAT GAGGAGGGC GCATGCCAG CCTTGCCCC ATCAACTGC
ACCCACTCC TGTGTGGAC CTGGATGAC AAGGGCTGC CCCGCCGAG CAGAGAGCC AGCCCTCTG
ACGTCCATC ATCTCTGCG GTGGTTGGC ATTCTGCTG GTCGTGGTC TTGGGGGTG GTCTTTGGG
ATCCTCATC TGA (SEQ ID NO:10)

FIG.6B

RHESUS MONKEY IMMUNIZATION STUDIES

IMMUNIZATION	DNA	DNA	DNA	DNA		Ad5	Ad5			
WEEK	0	8	12	16	23	27	31	35	40	44
RI-497										
A	3	0	0	0	10	n.t.	3	0	0	0
B	5	0	0	47	20	n.t.	3	0	5	0
C	5	15	45	50	60	n.t.	0	0	5	0
D	13	20	5	67	47	n.t.	10	0	5	0
E	10	0	0	15	25	n.t.	5	0	0	0
F	5	0	0	0	17	n.t.	13	8	8	0
G	8	0	0	57	20	n.t.	15	0	0	0
H	3	20	0	35	30	n.t.	0	0	5	0
J	8	0	0	75	37	n.t.	3	0	5	0
RI-503										
A	3	18	13	5	8	n.t.	3	5	5	0
B	0	13	13	3	5	n.t.	3	3	5	0
C	1	10	13	15	8	n.t.	3	3	3	3
D	4	8	13	5	8	n.t.	0	3	0	0
E	6	10	10	8	3	n.t.	3	13	8	18
F	4	13	33	13	10	n.t.	20	95	13	10
G	8	8	18	5	8	n.t.	0	3	3	0
H	4	15	23	15	10	n.t.	5	3	0	0
J	4	13	13	13	5	n.t.	3	3	3	0
RI-512										
A	3	0	23	2	0	n.t.	0	0	13	0
B	14	0	23	22	0	n.t.	43	65	65	15
C	20	0	30	17	3	n.t.	53	60	85	8
D	13	0	15	5	0	n.t.	0	0	8	0
E	24	0	0	2	0	n.t.	23	23	28	8
F	8	0	23	0	20	n.t.	303	473	535	145
G	21	0	13	7	0	n.t.	0	8	988	183
H	19	3	28	n.t.	8	n.t.	n.t.	10	8	3
J	13	3	0	n.t.	13	n.t.	n.t.	140	128	15
RI-520										
A	3	0	0	15	0	n.t.	0	3	2.5	0
B	0	3	0	0	0	n.t.	0	3	0	0
C	0	0	5	5	0	n.t.	3	3	0	0
D	0	8	17	10	7	n.t.	0	0	0	0
E	0	0	0	20	0	n.t.	33	3	5	35
F	0	5	10	10	0	n.t.	10	28	30	54
G	0	3	0	5	0	n.t.	0	3	2.5	0
H	0	3	0	5	0	n.t.	0	5	0	0
J	0	0	2	20	0	n.t.	0	0	0	e

FIG.7

IMMUNIZATION OF MICE WITH pV1J-HER2opt AND pV1J-HER2ECDTM.opt

	hNeu15.3	hNeu41
pVIJ-hHER2.opt	468	12
pVIJ-hHER2ECDTM.opt	655	92

FIG.8